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Accumulation of Heavy Metals by *Eisenia foetida* from Different animal dung and Kitchen wastes during Vermicomposting

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ABSTRACT:
Heavy metals concentrations observed in different animal dung with kitchen wastes and final vermicompost as well as earthworm body. Different heavy metals such as Cobalt, Chromium, Cadmium, Nickel, Lead and Arsenic (Co, Cr, Cd, Ni, Pb and As) accumulated by earthworm *Eisenia foetida* and after vermicomposting increased metals in earthworm body. There was significant decrease heavy metals concentration observed in all combinations after vermicomposting. The maximum decrease of Co was observed combination of cow dung + KW (0.023±0.006 mg/kg), Pb in horse dung control (0.008±0.006 mg/kg). The reduction of Cd and Cr was (0.043±0.004 and 0.084±0.003 mg/kg) in goat dung control and goat dung + KW respectively, The concentration of Ni in all combination of goat dung + KW (BDL) and As in all combination of sheep dung + KW (BDL). From present study it is clearly demonstrated that *E. foetida* significantly decrease the different heavy metals level in the final vermicompost during vermicomposting. This technology is useful for management of kitchen waste and reduction of heavy metals concentration in vermicompost by *E. foetida* resulting the production of safety food which protects the human health and environment.

Key words: Accumulation, Animal dung, *Eisenia foetida*, Health problems, Heavy metals, Kitchen wastes (KW) and Vermicomposting.

INTRODUCTION

In India and other developing countries generated large amount of kitchen/agricultural wastes. It causes a serious problem to environment and human health [1-2]. The microbial decomposition of animal agro/kitchen waste produced odor problem. Vermicomposting is one of the suitable ways for the management of biological waste by *E. foetida* [3-4]. It was reported that vegetable crop/wastes produced from the different polluted agriculture field of India have higher concentration of Cd, Ni, Cr, Pb etc. with respect to other [5-6]. Animal manure contains heavy metals and toxic metals added to soil through these manure could be entered to human body through the food chain [7-9]. Luo et al, [10] reported that vegetable wastes contain huge amount of Cd, Pb and Cr which ultimately reached into agricultural field. Heavy metals accumulation in fruit and vegetables by intake of plant from polluted soil makes it more susceptible [11]. The vegetable waste produced from market, discarded into the land fills, increase the heavy metals in the soil [12]. A possible way to utilize these wastes is by vermibiotechnology [13-14]. Langerweff [15] reported that cadmium is highly toxic metal to human being.

The heavy metals entered in the human body by ingestion of contaminated foodstuff specially grains, cereals and leafy vegetables. Lead is responsible for the damage liver, kidney and brain cells which cause ultimately death, in pregnant women cause miscarriage and cadmium is also causes acute and chronic toxicity symptom in human [16-19]. Brown, [20] reported that cobalt is responsible for the beer hearth syndrome in the human. Heavy metals are non essential and hazardous elements concern the chromium cause lung cancer. It can cause several respiratory irritation lung disease, cancers and kidney problem [21-22]. Chromium spreads the disease in human being, life breathing problem such as asthma, cough wheezing and skin contact can causes skin ulcer. Arsenic is carcinogens and causes cancer of skin, lung and liver. Heavy metals release naturally by erosion of rocks, volcanic activity, forest fire and artificially by many industries, paper mills, vehicles and human activities and it can release in large quantities directly effect the flora, fauna as well as human population [19]. Vermicompost decreased heavy metals and rich source of beneficial microorganisms, higher level of plant available nutrients and therefore may enhance soil fertility [23]. Increase in crop yield, soil nutrients status and nutrients uptake was reported due to application of vermicompost [24-25]. Earthworm *E. foetida* accumulate heavy metals in their body tissues during vermicomposting of kitchen wastes with different animal dung [26-28].

The aim of the present study is to investigate the level of heavy metal accumulated by earthworm *E. foetida* from initial vermibed and final vermicompost of different combination of animal dung with kitchen wastes. The heavy metal concentration is also estimated in the earthworm body before inoculation in the vermibeds and after vermicomposting.

MATERIALS AND METHODS

Collection of Wastes

Animal wastes (cow, buffalo, sheep, goat and horse dung) were collected from different farm houses and

kitchen wastes were collected from different part of Gorakhpur city and exposed to sun light for 5 to 10 days for removal of various harmful organism and noxious gases.

Collection of Earthworm

Earthworm *E. foetida* an epigeic species were cultured in laboratory. Temperature (20^0C - $30\ ^0$C), aeration and moisture (40% - 60%) were maintained for proper growth and development of earthworm.

Experimental setup for vermicomposting

The vermicomposting conducted on cemented earth surface by the method of Gupta, [29]. The different combinations of animal dung and kitchen waste in 1:1, 1:2 and 1:3 ratio were used for preparation of vermibeds. The size of each vermibed is 3m ×1m × 9cm. After formation of vermibed were inoculated 2kg of cultured *Eisenia foetida* in each bed. The beds were covered with jute pockets and moisten the beds daily up to 40 to 50 days for maintaining the moisture content. Each vermibed were manually turned over up to 3 weeks after one week interval. After 60 days granular tea like vermicompost appear on the surface of each bed. The prepared vermicomposts and inoculated earthworm were used for experiments.

Analysis of heavy metals in initial feed mixture and final vermicompost

The heavy metal content in the initial feed mixture and final vermicompost were measured by the method of Maboeta, [30]. About 1 gm of initial feed mixture and final vermicompost were subjected to digestion by adding excess of nitric acid (1:1) and were placed on hot plate and heated for 4 hours at 90 ^{0}C to 100 ^{0}C. Attentions were taken to ensure that simple did not dry out during digestion. After digestion sample will be poured into 100 ml flask through Whatman No 41 filter paper and injected into flame atomic absorption for determination of the heavy metal concentration.

Analysis of heavy metals in earthworm body

The heavy metals in the earthworm body tissue were digested using by the method of Katz and Jenneis [31]. Earthworm were individually dried, ground and burned to ash at high temperature. The ashes have placed in a test tube and about 10 to 15 ml of 55% nitric acid have added in it. Solution have left for 12 hrs at room temperature and heated again a temperature of 40 ^{0}C to 60 ^{0}C for 2 hrs and then at a temperature of 120 ^{0}C to 130 ^{0}C for one hrs. Once again heated the sample at 120 ^{0}C to 130 ^{0}C and 1 ml of 70% perchloric acid has added. The samples have allowed cool before adding 5 ml of distilled water. Again heated sample up to 130 ^{0}C until white fumes liberated and allowed to cool finally, before being micro filtered. Filtered the solution through filter paper Whatman No 41 into 100 ml flasks. This solution was taken for measurement of heavy metals content in earthworm body by flame atomic absorption.

STATISTICAL ANALYSIS

All the data are mean ±SD of 6 replicates. Students‘t’ test was applied to determine the significant ($P<0.05$) difference between initial and final vermicompost [32].

RESULTS AND DISCUSSION

There was significant reduction ($P<0.05$‘t’ test) in heavy metals (Co, Cr, Cd, Ni, Pb and As) observed in final vermicompost of different animal dungs and kitchen wastes with respect to initial feed mixture (Tables 1, 2). The significant reduction of Co was observe in combination of cow dung with kitchen waste (0.023±0.006 mg/kg). The reduction of Co may due to the accumulation of Co in body tissue of earthworm [27, 33]. The goat dung with kitchen wastes have maximum reduction in the level of Cr (0.084±0.003 mg/kg) and Pb was observed in the combination of horse dung control in the level of (0.008±0.006 mg/kg). Table-2, shows that the concentration of Ni in combination of goat dung control and goat dung with kitchen waste was below detectible limit. The concentration of Cd in goat dung with kitchen waste was (0.043±0.004 mg/kg). The high rate of accumulation capability of heavy metals may responsible for these results [27-28, 34]. The maximum significant reduction of As in combination of cow and sheep dung with kitchen waste was BDL. It is clear that the reduction of heavy metals concentration of different animal dung with kitchen wastes was directly related to earthworm activity during wastes decomposition system [35]. Suthar et al., [33] reported that different metal content in final vermicompost are related to the different rate of physiological metabolism of earthworm. It is possible that vermic activity, growth and development of earthworm *E. foetida* are better in combination of kitchen waste with cow and sheep dung [36]. The high rate of vermic activity may be causes higher rate of accumulation of heavy metals from wastes during vermicomposting [27]. The organic matter ingested by earthworm under goes different chemical and microbial changes during vermicomposting [37]. Significantly changed fraction distribution and bioavailability of these heavy metals may be due to the accumulation in earthworm body tissue during vermic activity [38-40]. Morgan and Morgan [41] reported that accumulation of metals (Cd, Cu, Pb, Zn and Ca) by earthworm species during vermicomposting. Earthworm *E. foetida* have ability to bioaccumulation the heavy metals in their body from municipal solid wastes [34, 42].

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Table 1. Concentration of heavy metals (mg/kg) in combinations of different animal dung with kitchen wastes in initial feed mixture and final vermicompost.

Vermicompost	Ratio	Heavy metals (mg/kg)					
		Co		Cr		Pb	
		Initial	Final	Initial	Final	Initial	Final
K W Control	-	0.054 ± 0.006	0.014 ± 0.003*	0.816 ± 0.003	0.159 ± 0.005*	0.345 ± 0.005	0.120 ± 0.004*
Buffalo							
Dung	-	0.029 ± 0.008	0.008 ± 0.004*	1.064 ± 0.003	0.216 ± 0.003*	0.782 ± 0.006	0.193 ± 0.003*
Dung + KW	1:1	0.029 ± 0.003	0.010 ± 0.003*	0.994 ± 0.008	0.144 ± 0.002*	0.598 ± 0.004	0.081 ± 0.007*
	1:2	0.069 ± 0.003	0.028 ± 0.002*	0.872 ± 0.002	0.123 ± 0.003*	0.653 ± 0.003	0.096 ± 0.005*
	1:3	0.290 ± 0.006	0.036 ± 0.003*	0.786 ± 0.006	0.062 ± 0.003*	1.692 ± 0.004	0.287 ± 0.002*
Cow							
Dung	-	BDL	BDL	0.848 ± 0.003	0.124 ± 0.003*	0.199 ± 0.002	0.078 ± 0.003*
Dung + KW	1:1	BDL	BDL	0.766 ± 0.004	0.081 ± 0.004*	0.193 ± 0.005	0.054 ± 0.005*
	1:2	BDL	BDL	0.783 ± 0.009	0.090 ± 0.005*	0.224 ± 0.006	0.062 ± 0.006*
	1:3	0.069 ± 0.001	0.023 ± 0.006*	0.802 ± 0.007	0.108 ± 0.004*	0.369 ± 0.005	0.089 ± 0.002*
Goat							
Dung	-	0.022 ± 0.002	0.007 ± 0.003*	0.472 ± 0.008	0.109 ± 0.004*	0.184 ± 0.004	0.062 ± 0.003*
Dung + KW	1:1	0.037 ± 0.006	0.018 ± 0.004*	0.443 ± 0.005	0.084 ± 0.003*	0.175 ± 0.007	0.057 ± 0.004*
	1:2	0.061 ± 0.005	0.020 ± 0.002*	0.643 ± 0.004	0.105 ± 0.005*	0.336 ± 0.003	0.088 ± 0.004*
	1:3	0.152 ± 0.003	0.024 ± 0.005*	0.885 ± 0.003	0.112 ± 0.004*	0.655 ± 0.002	0.154 ± 0.002*
Horse							
Dung	-	0.018 ± 0.002	BDL *	0.795 ± 0.003	0.109 ± 0.005*	0.024 ± 0.004	0.008 ± 0.006*
Dung + KW	1:1	0.024 ± 0.004	0.008 ± 0.006*	0.757 ± 0.004	0.102 ± 0.007*	0.093 ± 0.005	0.031 ± 0.003*
	1:2	0.047 ± 0.005	0.019 ± 0.003*	0.872 ± 0.006	0.122 ± 0.004*	0.158 ± 0.004	0.054 ± 0.004*
	1:3	0.107 ± 0.006	0.037 ± 0.004*	0.808 ± 0.007	0.118 ± 0.004*	0.783 ± 0.006	0.102 ± 0.004*
Sheep							
Dung	-	0.055 ± 0.003	0.017 ± 0.004*	0.819 ± 0.005	0.113 ± 0.007*	0.748 ± 0.005	0.106 ± 0.004*
Dung + KW	1:1	0.155 ± 0.003	0.044 ± 0.004*	0.830 ± 0.005	0.124 ± 0.005*	0.593 ± 0.003	0.103 ± 0.004*
	1:2	0.256 ± 0.007	0.035 ± 0.002*	0.608 ± 0.008	0.095 ± 0.004*	0.792 ± 0.004	0.101 ± 0.005*
	1:3	0.281 ± 0.008	0.057 ± 0.006*	0.770 ± 0.006	0.102 ± 0.006*	0.886 ± 0.008	0.124 ± 0.003*

KW = Kitchen wastes, BDL= below detectable limit
Each value is the Mean ± SD of six replicates.
***** Significant P<0.05 "t" test between initial feed mixture and final vermicompost.

Table 2. Concentration of heavy metals (mg/kg) in combinations of different animal dung with kitchen wastes in initial feed mixture and final vermicompost.

Vermicompost	Ratio	Heavy metals (mg/kg)					
		Ni		Cd		As	
		Initial	Final	Initial	Final	Initial	Final
K W Control	-	0.174 ± 0.003	0.035 ± 0.004*	0.126 ± 0.005	0.059 ± 0.006*	0.167 ± 0.003	0.036 ± 0.004*
Buffalo							
Dung	-	0.844 ± 0.005	0.118 ± 0.005*	0.398 ± 0.006	0.061 ± 0.004*	0.168 ± 0.005	0.031 ± 0.005*
Dung + KW	1:1	0.908 ± 0.005	0.153 ± 0.003*	0.383 ± 0.003	0.057 ± 0.002*	0.178 ± 0.004	0.043 ± 0.006*
	1:2	0.842 ± 0.003	0.147 ± 0.004*	0.258 ± 0.004	0.052 ± 0.006*	0.216 ± 0.006	0.046 ± 0.004*
	1:3	0.768 ± 0.003	0.136 ± 0.004*	0.174 ± 0.004	0.034 ± 0.003*	0.269 ± 0.003	0.052 ± 0.004*
Cow							
Dung	-	0.116 ± 0.003	0.028 ± 0.003*	0.158 ± 0.005	0.028 ± 0.005*	0.057 ± 0.004	BDL *
Dung + KW	1:1	0.105 ± 0.007	0.026 ± 0.004*	0.150 ± 0.006	0.023 ± 0.003*	0.052 ± 0.002	BDL *
	1:2	0.134 ± 0.005	0.033 ± 0.002*	0.174 ± 0.003	0.049 ± 0.002*	0.067 ± 0.003	BDL *
	1:3	0.289 ± 0.004	0.057 ± 0.003*	0.189 ± 0.005	0.060 ± 0.003*	0.152 ± 0.002	0.041 ± 0.004*
Goat							
Dung	-	0.107 ± 0.005	BDL *	0.391 ± 0.003	0.108 ± 0.005*	0.118 ± 0.005	0.037 ± 0.003*
Dung + KW	1:1	0.086 ± 0.002	BDL *	0.114 ± 0.003	0.043 ± 0.004*	0.076 ± 0.003	0.032 ± 0.004*
	1:2	0.094 ± 0.005	BDL *	0.178 ± 0.001	0.055 ± 0.004*	0.079 ± 0.002	0.029 ± 0.003*
	1:3	0.100 ± 0.003	BDL *	0.218 ± 0.002	0.092 ± 0.006*	0.108 ± 0.004	0.022 ± 0.003*
Horse							
Dung	-	0.106 ± 0.003	0.029 ± 0.005*	0.483 ± 0.001	0.096 ± 0.003*	0.099 ± 0.006	0.025 ± 0.006*
Dung + KW	1:1	0.117 ± 0.003	0.035 ± 0.006*	0.384 ± 0.004	0.103 ± 0.003*	0.236 ± 0.005	0.048 ± 0.005*
	1:2	0.168 ± 0.004	0.032 ± 0.008*	0.174 ± 0.004	0.038 ± 0.005*	0.142 ± 0.004	0.036 ± 0.006*
	1:3	0.254 ± 0.005	0.108 ± 0.002*	0.135 ± 0.003	0.032 ± 0.004*	0.200 ± 0.003	0.051 ± 0.003*
Sheep							
Dung	-	0.112 ± 0.005	0.055 ± 0.005*	0.288 ± 0.004	0.067 ± 0.003*	0.061 ± 0.004	BDL *
Dung + KW	1:1	0.104 ± 0.003	0.032 ± 0.003*	0.133 ± 0.006	0.059 ± 0.005*	0.052 ± 0.003	BDL *
	1:2	0.133 ± 0.003	0.028 ± 0.004*	0.158 ± 0.008	0.032 ± 0.004*	0.053 ± 0.005	BDL *
	1:3	0.276 ± 0.002	0.066 ± 0.002*	0.274 ± 0.005	0.041 ± 0.006*	0.040 ± 0.003	BDL *

KW = Kitchen wastes, BDL= below detectable limit
Each value is the Mean ± SD of six replicates.
***** Significant P<0.05 "t" test between initial feed mixture and final vermicompost.

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Table 3.Concentration of heavy metals (mg/kg) in different combination of animal dung with Kitchen wastes in final earthworm body after vermicomposting.

Vermicompost	Ratio	Heavy metals (mg/kg)					
		Co	Cr	Pb	Ni	Cd	As
During inoculation Earthworm body (Control)	-	6.734 ± 0.004	114.515 ± 0.006	9.438 ± 0.005	6.339 ± 0.002	61.645 ± 0.004	9.450 ± 0.003
After vermicomposting							
KW	-	6.758 ± 0.002*	115.031 ± 0.004*	9.562 ± 0.002*	6.446 ± 0.003*	61.703 ± 0.003*	9.512 ± 0.004*
Buffalo							
Dung		6.749 ± 0.003*	115.187 ± 0.002*	9.940 ± 0.003*	7.004 ± 0.005*	61.851 ± 0.003*	9.578 ± 0.003*
Dung + KW	1:1	6.746 ± 0.006*	115.024 ± 0.003*	9.733 ± 0.006*	7.126 ± 0.004*	61.835 ± 0.003*	9.571 ± 0.004*
	1:2	6.775 ± 0.004*	115.017 ± 0.002*	9.842 ± 0.005*	7.104 ± 0.003*	61.831 ± 0.005*	9.616 ± 0.003*
	1:3	6.901 ± 0.003*	114.981 ± 0.004*	10.039 ± 0.004*	6.848 ± 0.003*	61.857 ± 0.005*	9.648 ± 0.002*
Cow							
Dung	-	6.734 ± 0.003*	114.905 ± 0.006*	9.531 ± 0.003*	6.324 ± 0.003*	61.756 ± 0.004*	9.485 ± 0.003*
Dung + KW	1:1	6.734 ± 0.002*	115.005 ± 0.005*	9.518 ± 0.004*	6.338 ± 0.006*	61.741 ± 0.002*	9.482 ± 0.005*
	1:2	6.734 ± 0.004*	115.021 ± 0.003*	9.553 ± 0.005*	6.414 ± 0.003*	61.762 ± 0.001*	9.503 ± 0.005*
	1:3	6.781 ± 0.003*	115.072 ± 0.003*	9.649 ± 0.003*	6.488 ± 0.003*	61.791 ± 0.003*	9.542 ± 0.004*
Goat							
Dung	-	6.745 ± 0.003*	114.837 ± 0.003*	9.508 ± 0.006*	6.428 ± 0.004*	61.894 ± 0.004*	9.532 ± 0.002*
Dung + KW	1:1	6.748 ± 0.004*	114.724 ± 0.004*	9.536 ± 0.005*	6.391 ± 0.002*	61.709 ± 0.006*	9.486 ± 0.003*
	1:2	6.770 ± 0.003*	114.919 ± 0.003*	9.644 ± 0.004*	6.394 ± 0.008*	61.753 ± 0.003*	9.492 ± 0.002*
	1:3	6.843 ± 0.003*	115.124 ± 0.004*	9.883 ± 0.004*	6.397 ± 0.004*	61.812 ± 0.003*	9.525 ± 0.007*
Horse							
Dung	-	6.747 ± 0.003*	115.021 ± 0.002*	9.451 ± 0.004*	6.397 ± 0.002*	61.945 ± 0.003*	9.504 ± 0.003*
Dung + KW	1:1	6.745 ± 0.005*	115.005 ± 0.003*	9.493 ± 0.003*	6.401 ± 0.006*	61.762 ± 0.006*	9.548 ± 0.007*
	1:2	6.763 ± 0.004*	115.137 ± 0.003*	9.592 ± 0.004*	6.428 ± 0.003*	61.793 ± 0.005*	9.592 ± 0.005*
	1:3	6.787 ± 0.002*	115.158 ± 0.004*	10.045 ± 0.002*	6.542 ± 0.004*	61.698 ± 0.007*	9.607 ± 0.002*
Sheep							
Dung	-	6.771 ± 0.004*	115.001 ± 0.004*	9.886 ± 0.003*	6.426 ± 0.004*	61.868 ± 0.003*	9.491 ± 0.003*
Dung + KW	1:1	6.778 ± 0.003*	114.975 ± 0.003*	9.873 ± 0.004*	6.391 ± 0.002*	61.751 ± 0.004*	9.485 ± 0.005*
	1:2	6.824 ± 0.004*	114.993 ± 0.002*	9.939 ± 0.002*	6.409 ± 0.007*	61.768 ± 0.003*	9.488 ± 0.004*
	1:3	6.862 ± 0.002*	115.075 ± 0.006*	10.062 ± 0.003*	6.442 ± 0.007*	61.853 ± 0.002*	9.475 ± 0.004*

KW = Kitchen wastes
Each value is the Mean ± SD of six replicates.
***** Significant P<0.05 "t" test between earthworm body before inoculation in vermibeds and after vermicomposting.

Heavy metals (Co, Cr, Cd, Ni, Pb and As) concentration in the body of *E. foetida* was significantly increase after the vermicomposting of all combination of different animal dung with kitchen waste (Table-3). The maximum significant increase content of Co, Ni and As was observe in the body of earthworm, procured from combination of buffalo dung with kitchen waste (6.901±0.003 mg/kg), (7.126±0.004 mg/kg) and (9.648±0.002 mg/kg). Cr was significantly increase in the earthworm body obtain from combination of buffalo dung control (155.187±0.002 mg/kg). Pb and Cd was maximum in the earthworm body obtain from combination of sheep dung with kitchen waste (10.062±0.003 mg/kg) and horse dung control (61.945±0.003 mg/kg) respectively. The cobalt, cadmium, nickel and arsenic easy accumulation in earthworm body during vermicomposting of different animal dung with kitchen waste. Leonard and Dolfing [43] reported that accumulation of Cd by earthworm species *E. foetida* during vermic activity.

CONCLUSION

There was significant increase of heavy metals accumulation in their body tissue of *E. foetida* whereas decreased heavy metals level in final vermicompost of different animal dung (cow, buffalo, sheep, goat and horse dung) with kitchen wastes and the combination of buffalo dung with kitchen waste *E. foetida* have maximum accumulation of heavy metals in their body. In combination of buffalo dung with kitchen waste more time better activity of earthworm than other combination of animal dung with kitchen waste because it have low concentration of heavy metals in final vermicompost which good for human health and environment.

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